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New data on the acanthocephalan *Neoechinorhynchus villoldoi* Vizcaíno, 1992 (Neoechinorhynchidae: Acanthocephala), based on specimens found in *Austrolebias bellottii* (Steindachner, 1881) (Rivulidae: Cyprinodontiformes) from Punta Indio, Argentina

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Abstract

In a survey of parasites on the killifish, *Austrolebias bellottii* from Punta Indio and Magdalena, Argentina, the acanthocephalan *Neoechinorhynchus villoldoi* was found. This parasite had not been recorded since 1992 when it was described for the first time parasitizing *Corydoras paleatus* (Jenyns, 1842). The type material of *N. villoldoi* was examined, and with addition of the new specimens, the measurements range was extended. This is the first record of an acanthocephalan in the annual fish, *A. bellottii.*

Key words

Metazoan parasite; new record; annual fishes; pampa; temporary ponds.

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Introduction

Data on metazoan parasites of South American annual killifish is scarce. Delgado and Garcia (2015) reported 2 larvae of *Contracaecum* spp. nematode in *Austrolebias* spp. from Uruguay, and Luque et al. (2011) mentioned the presence of the nematode *Hedruris iheringi* Pereira & Vaz, 1933 in *Austrolebias bellottii* (Steindachner, 1881) from Brazil. Due to a lack of information in Argentina, we began to search for parasites on *A. bellottii*.

Among genera of the Neotropical fish fauna, the South American killifish genus *Austrolebias* Costa, 1998 are the most numerous annual fishes distributed in southern Bolivia, southern Brazil, Paraguay, northeastern Argentina and Uruguay with 44 valid species (Costa 2010, Loureiro et al. 2011, Nielsen and Pillet 2015). The genus *Austrolebias* inhabits temporary wetlands formed during rainy periods and die when the ponds dry out (Costa 2003, Costa 2006). Most species bury their eggs in the substrate, the embryos survive through dry periods in a diapause inside the resistant eggs and hatch at the beginning of the

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Figures 1, 2. Photography's of the *Austrolebias bellottii* **1.** Male, SL 3.8 cm. **2:** female, SL 3.4 cm.

rainy season (Wourms 1972, Podrabsky and Hand 1999). The fishes grow fast, mostly in the first months of their life, reach the sexual maturity early and begin to spawn (Walford and Liu 1965, Gonzalves et al. 2011).

The short life cycle, the ecological importance, the aquarist interest of this fish, and the scant information on metazoan parasites in killifishes moved us to study the acanthocephalan *Neoechinorhynchus villoldoi* Vizcaíno, 1992. Based on reexamination of the type material from

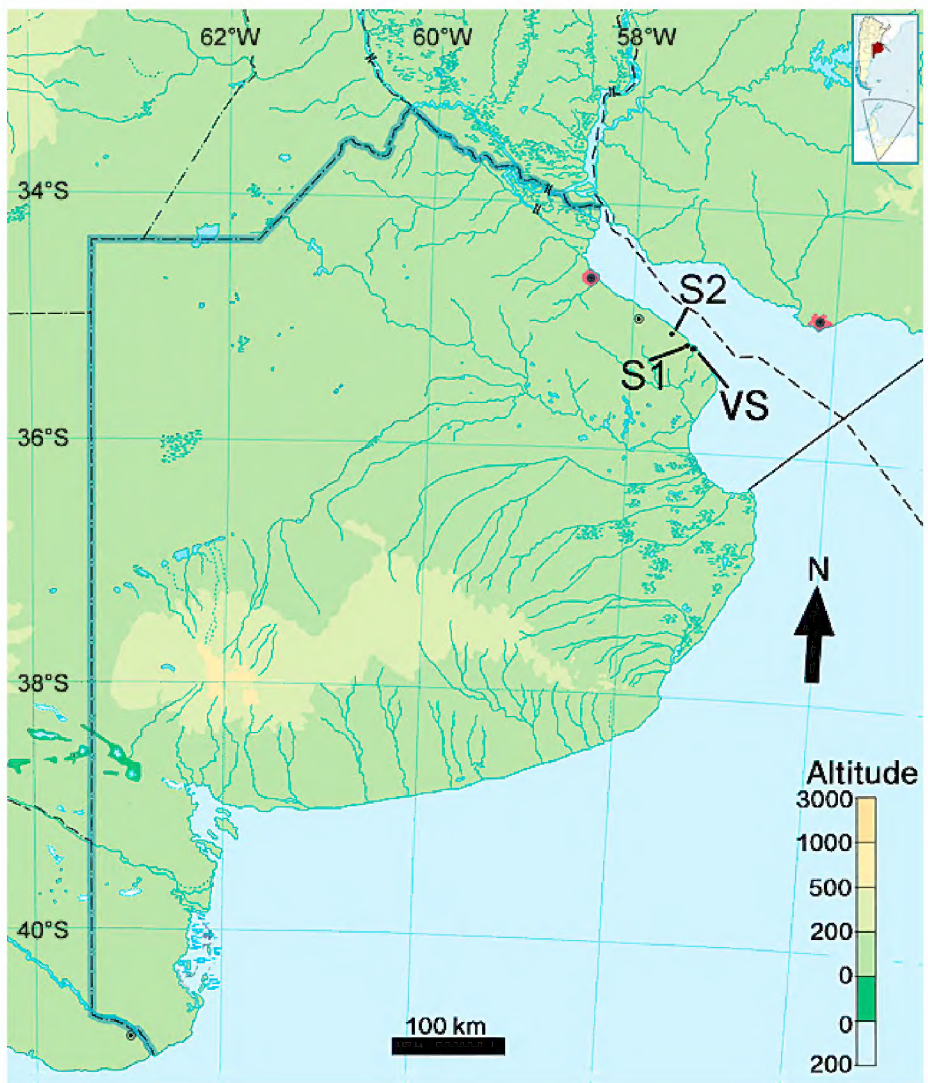


Figure 3. Map of Buenos Aires province, Argentina, with the 2 sample sites, and Villoldo stream (VS).

Figures 4, 5. Sample sites. **4.** Site 1, Punta Indio. **5.** Site 2, Magdalena.

Corydoras paleatus (Jenyns, 1842) and the new specimens found parasitizing *A. bellottii*, we extend the range of measurements of this parasite. This is the first record of *N. villoldoi* on *A. bellottii*.

Methods

Fishes (Figs 1, 2) were sampled between October and November 2015 from 2 temporary puddles separated from each other by 38 km (Fig. 3). The puddles (Figs 4, 5) were selected randomly and named: site 1 (Punta Indio, 35°18.932′ S, 057°13.176′ W) and site 2 (Magdalena, 35° 04.865′ S, 057°31.627′ W). The collections were made with a frame of 1 × 0.50 m and a net-mesh of 0.5 × 0.5 cm. The specimens were transported to the lab in plastic bags with oxygenated water from the collection sites. The fishes were sacrificed by medullar section. The viscera were examined for parasites under a binocular microscope. The acanthocephalans found were cleared as temporary mounts with Lacto-phenol or stained with dehydrated chlorhidric carmin mounted in Canada balsam, and studied under an Olympus Bx51 microscope (Tokyo, Japan). The drawings were made with a drawing tube. The structures were photographed with an AmScope MU 1000 10 MP digital camera (USA) attached to the microscope and measured using ImageJ software (Schneider et al. 2012). The mean followed by minimum and maximum values in parenthesis are given in micrometers (µm), unless stated otherwise. Figures 1 and 2 were photographed with a Canon 7D camera with a Canon 100 mm 2.8 macro lens and Figures 4 and 5 with a

Table 1. Number of specimens of *Austrolebias bellottii* examined (*N*). Prevalence of infection, mean intensity and mean abundance of *Neoechinorhynchus villoldoi* in sample sites.

	N	Prevalence of infection	Mean intensity	Mean abundance
Punta Indio (sample site 1)	10	80%	3.13	2.5
Madariaga (sample site 2)	22	0%	0	0

Canon 50 mm 1.8 Macro lens. The vouchers were deposited in the Helminthological Collection of the Museo de La Plata, Argentina, under the number MLP-He 7150.

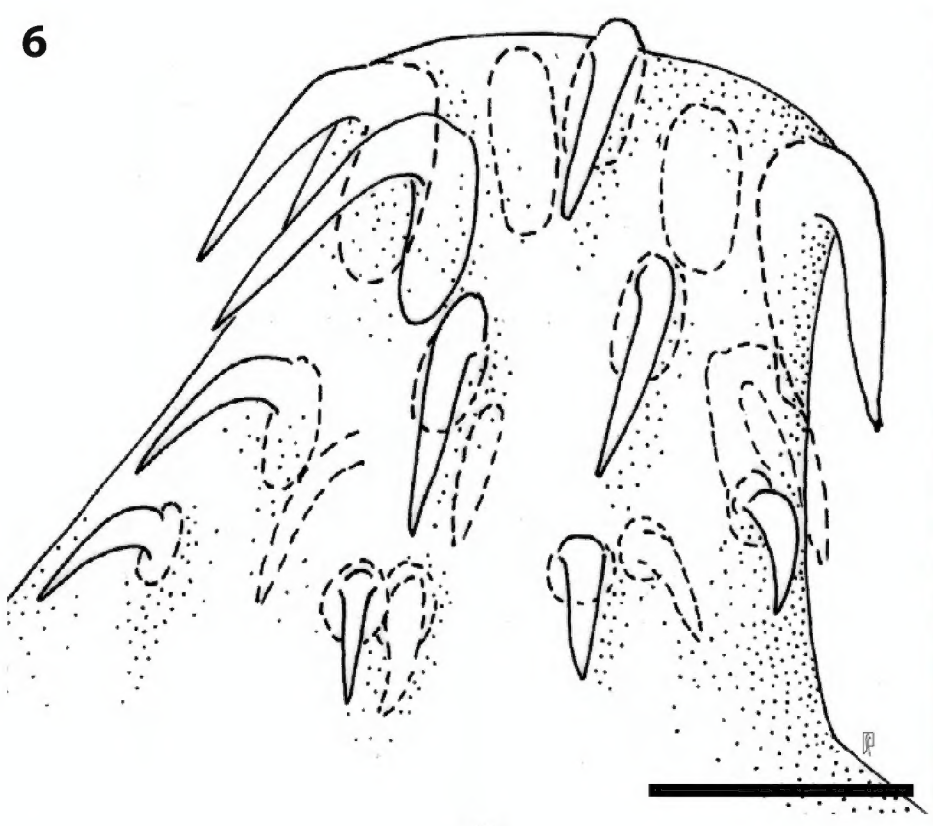
Examined material included all the type series of *Neoechinorhynchus villoldoi*, 1537 C, 1539 C, 1541-44 C and 1547-50 C, in the Helminthological Collection of Museo de la Plata.

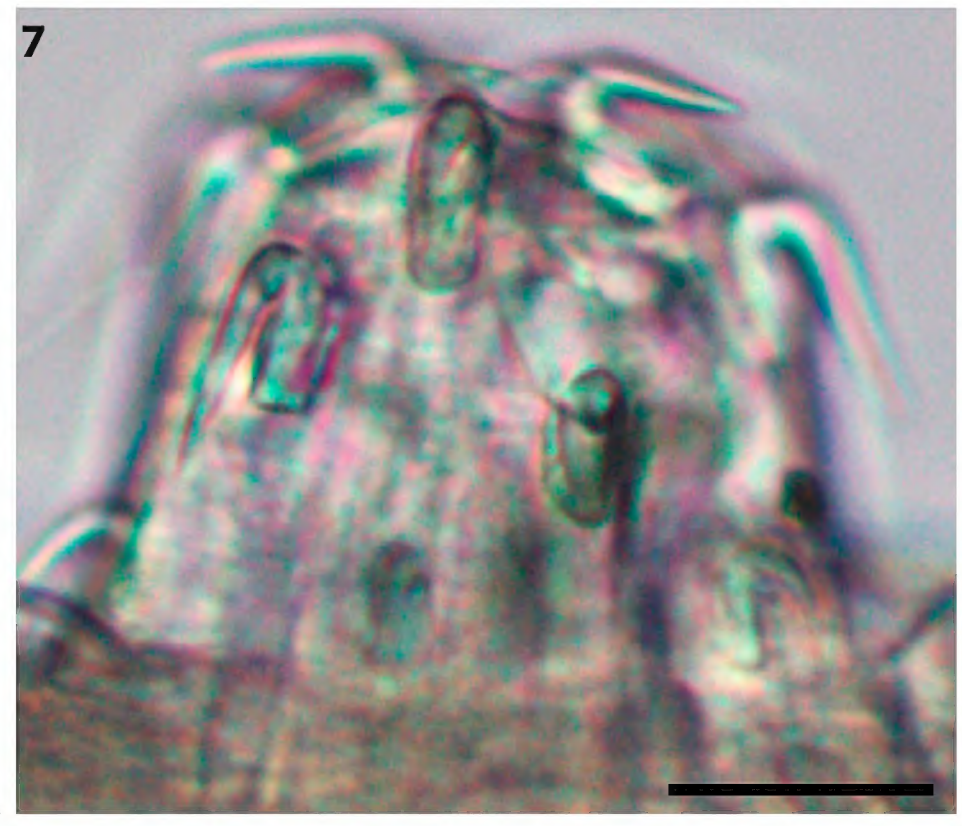
Results

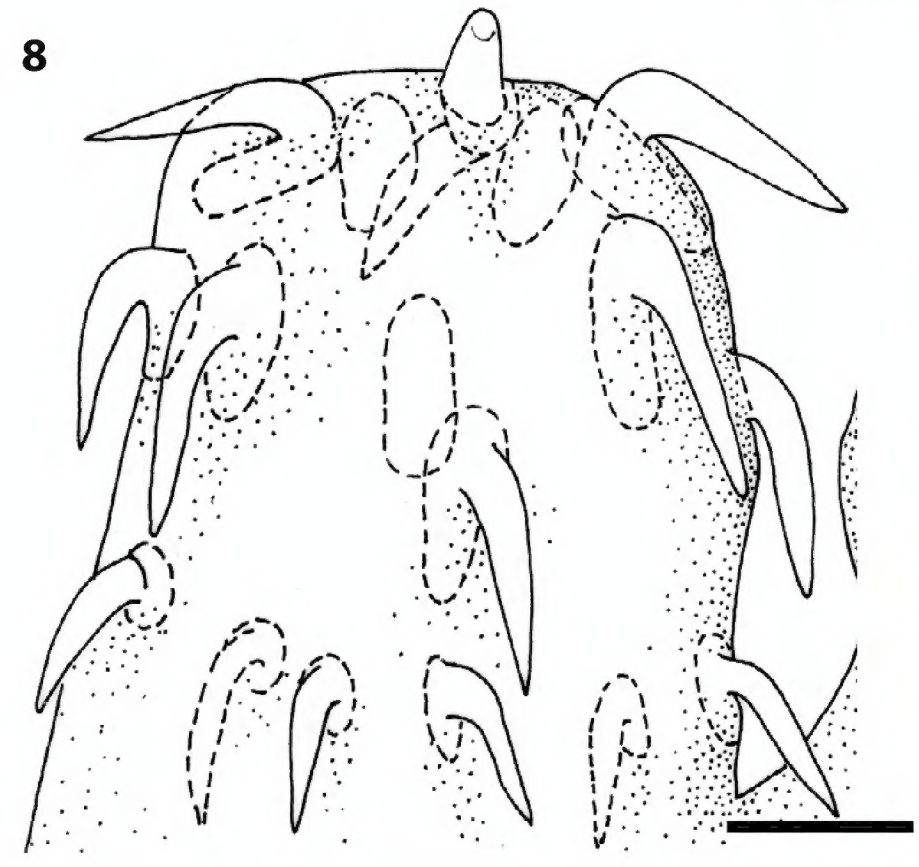
The prevalence, median intensity and abundance of *N. villoldoi* from *A. bellottii* are provided in Table 1.

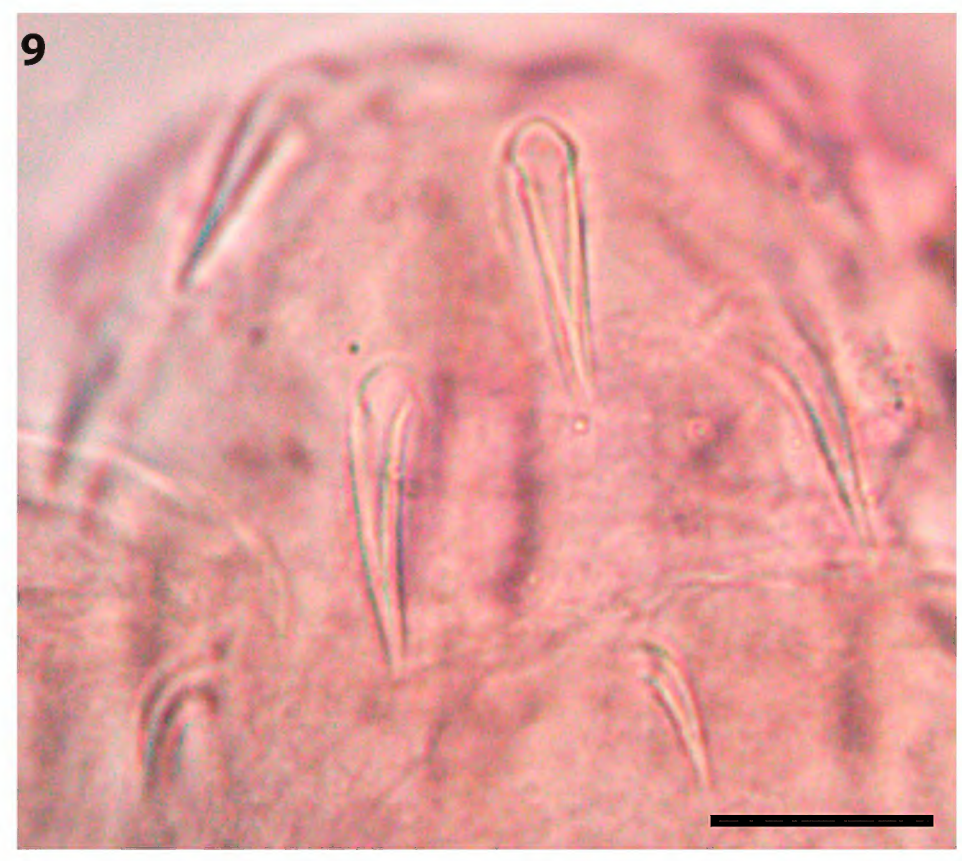
Description of *N. villoldoi* found in *A. bellottii*. Small, cylindrical, with gigantic hypodermic nuclei 1 or 2 dorsal and 1–3 ventral (not drawn), posterior end rounded. Genital pore subterminal in both sexes. Small proboscis, cylindrical, 18 hooks in 3 circles of 6 hooks each. First 2 circles with similar hooks lengths; third circle of hooks significantly smaller (Figs 6–9). Nerve ganglion posterior to the receptacle of the proboscis. Lemnisci short, subequal, barely longer than the receptacle of the proboscis.

Male (Figs 10, 11) shorter than female, reproductive system consisting of 2 contiguous testicles, cement gland syncytial, rounded reservoir of cement, elongated Säfftigen's pouch. Copulatory pouch spade-shaped (Figs 12, 13).

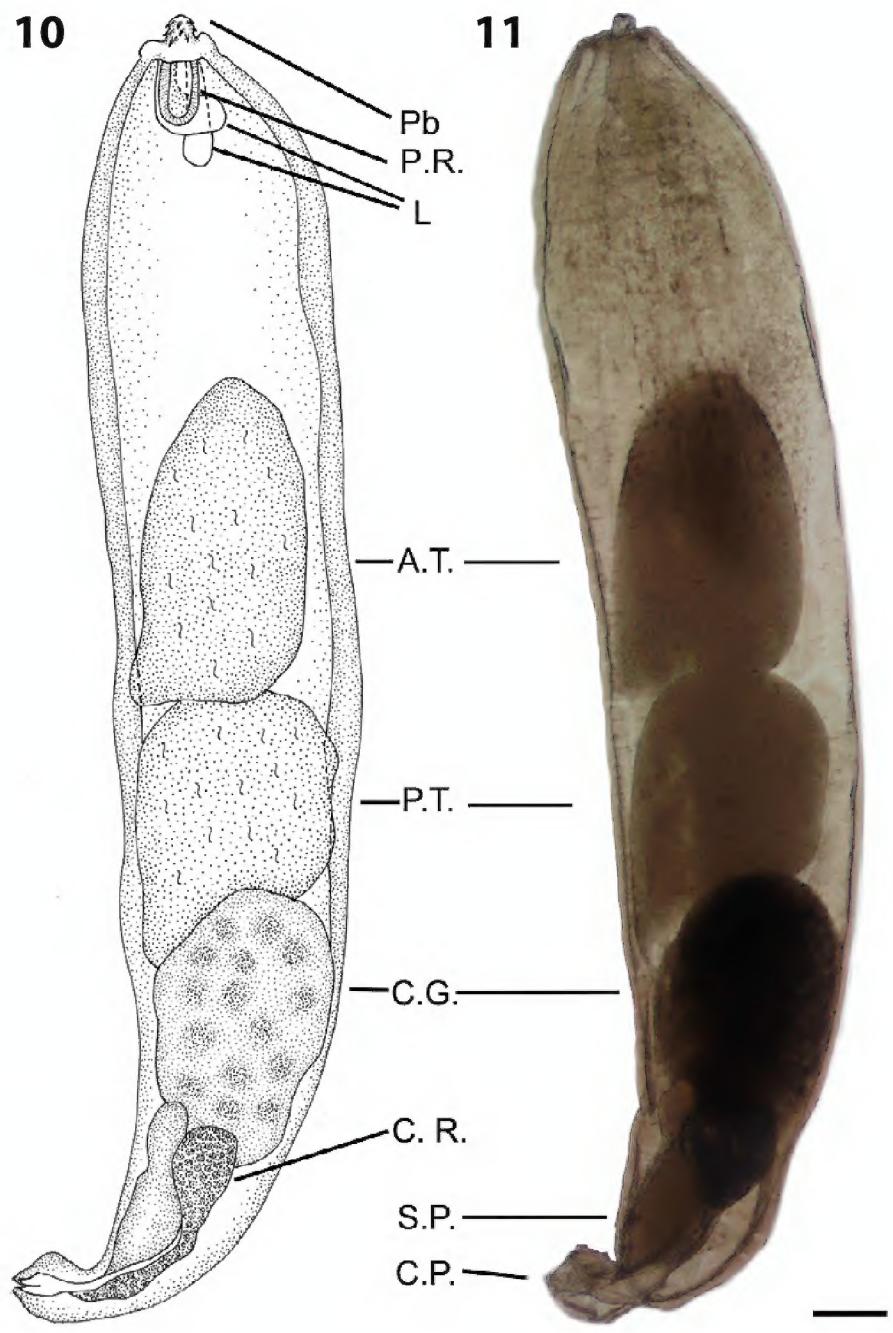








Figures 6–9. Proboscis of *Neoechinorhynchus villoldoi* found in *Austrolebias bellottii*. **6, 7.** Male proboscis. . **8, 9.** Female proboscis**; 9**, stained with chloridric carmin. Scale bars: Figs 6, 7, 9 = 20 µm, Fig. 8 = 16 µm.



Figures 10, 11. Entire male specimen of *Neoechinorhynchus villoldoi*. Abbreviations: AT = anterior testicle, CP = copulatory pouch, CG = cement gland, CR. = cement reservoir, L = lemnisci, Pb = proboscis, PT = posterior testicle, PR = proboscis receptacle, S P = Säfftigen's pouch. Scale bar = 200 µm.

Female (Figs 14–17) 3–4 × larger than the males, reproductive system includes uterine bell, selector of eggs, utero and vagina. Eggs elongated (Fig. 18). Measurements of male and female structures provided in Table 2.

Discussion

Neoechinorhynchus villoldoi is the first acanthocephalan found in a killifish of the genus *Austrolebias*. This is the second record for this acanthocephalan since the discovery of *N. villoldoi* by Vizcaíno (1992).

Our examination of the type series material deposited by Vizcaíno (1992) of *N. villoldoi* have similar measurements with the acanthocephalans found now in *A. bellottii*, but some minor differences can be distinguished. The type material included mostly juvenile females (only one mature with eggs) and males in poor conservation stage, and this could account for why some of the original measurements have a wide range.

The presence of *N. villoldoi* in this newly recorded host, *A. bellottii*, could be attributed to: (a) the proximity to the type locality or (b) the similarity in the host feeding habits.

Site 1 at Punta Indio is 7 km away from Villoldo stream (the type locality of *N. villoldoi*) but 35 km from site 2. The distance between sites could be one of the reasons for the absence of this parasite in fishes from Magdalena (site 1).

Corydoras paleatus (the type host) and *A. bellottii* are fishes with different habitat but perhaps they prey over the same food item. The diets of both species are represented mainly by microcrustaceans (Cladocera, Copepoda, and Ostracoda), and immature aquatic insects (Chironomidae) (Escalante 1983, Grosman 2002, Laufer 2009, Keppeler 2014). Better knowledge of the life cycle of *N. villoldoi* could clarify if this parasite uses 1, 2 or more intermediate hosts, and if both fishes prey over the same or different intermediate host?"

Knowledge of the parasite fauna of *A. bellottii* is important because this fish forms a link between micro- and macroecosystems. Killifishes mainly feed on microcrustaceans and immature aquatic insects, and killifishes serve as food for aquatic birds (Keppeler et al. 2016). They are also important fishes for aquarists due to the high number of species, bright colors, size and the relatively easy breeding in captivity.

There are many species of *Austrolebias,* and the genus has a wide distribution, and therefore we suspect that our record *N. villoldoi* in *A. bellottii* is only the first of a several new records (or new species) of adult metazoan parasites and larval stages waiting for to be discovered in these "killis".

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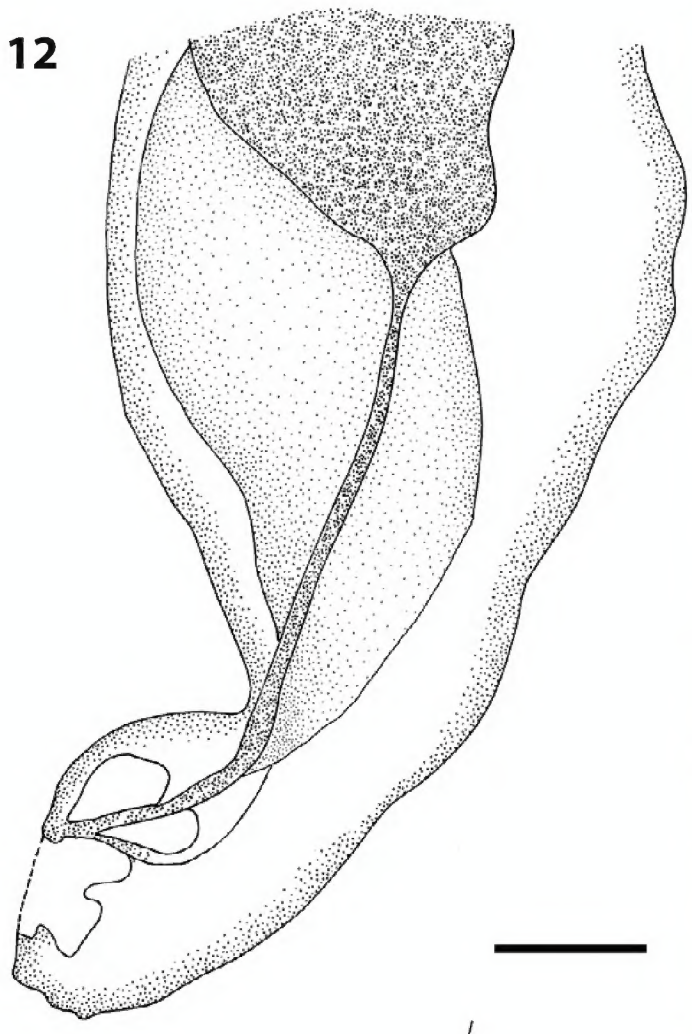
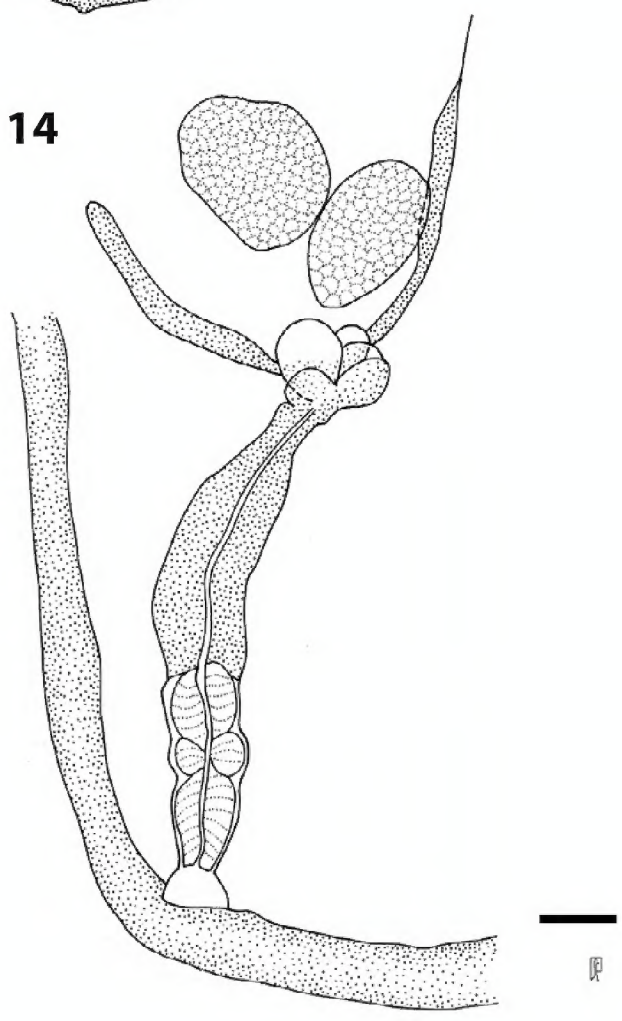
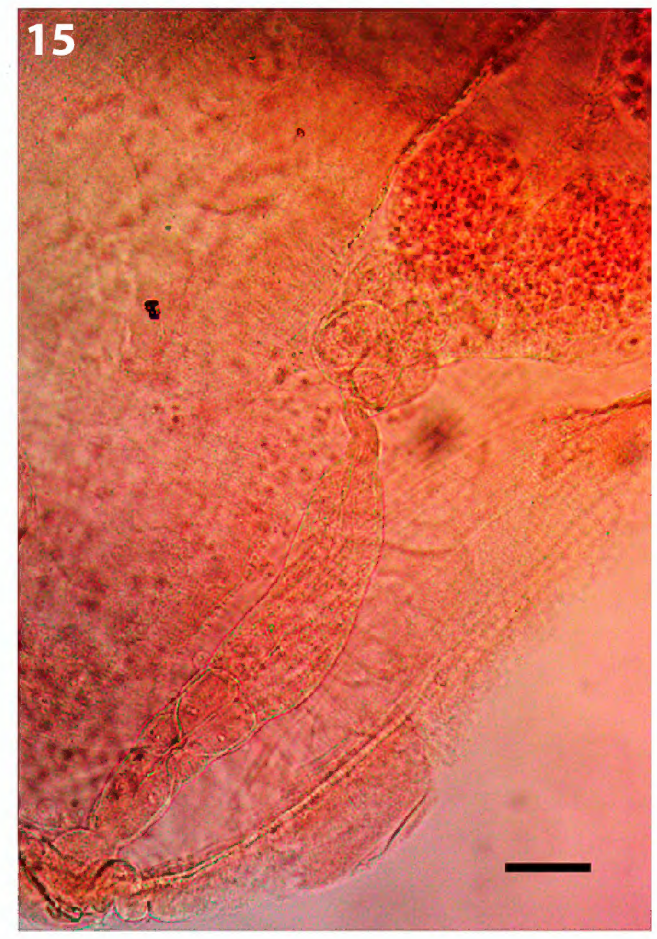
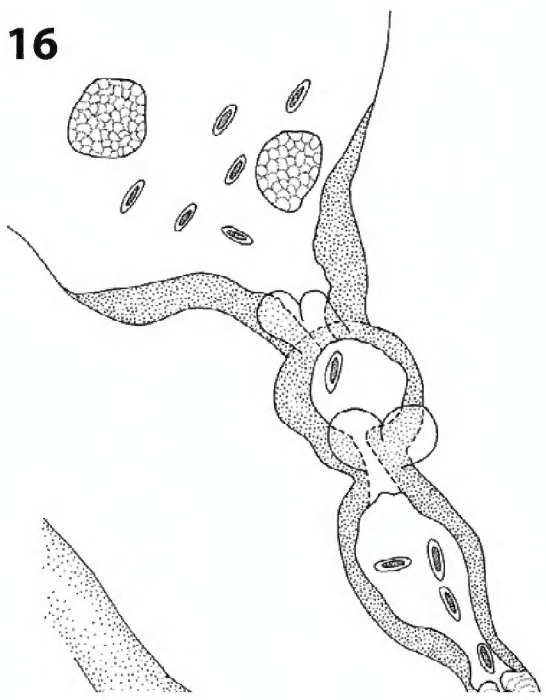
Authors' Contributions

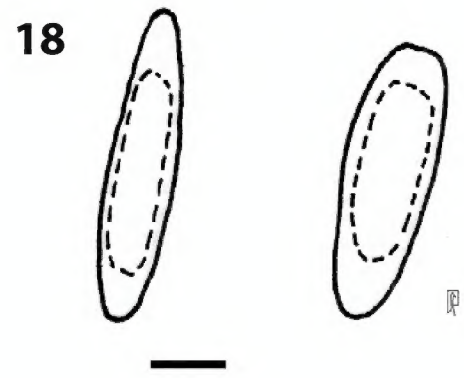
MMM identified the acanthocephalan, measured them, and wrote the text. JB collected the fishes and parasites and stained the acantocephalan. IG collected the samples, determined the fishes, and wrote the text (the part on the fishes). SP found the sample site, collected the samples, and photographed the male and female of *A. bellottii* and sample sites. SM identified the acanthocephalan, wrote the text.











Figures 14–18. Female reproductive of *Neoechinorhynchus villoldoi* found in *Austrolebias bellottii* and eggs. **14, 15** Juvenile female. **16, 17.** Reproductive female. **18.** Eggs. Abbreviations: CR = cement reservoir, SP = Säfftigen's pouch., Scale bars: Figs 14–15, 17 = 40 µm, Fig. 16 = 50 µm, Fig. 18 = 8 µm

Table 2. Comparison of *Neoechinorhynchus villoldoi* measurements between males and females of *Austrolebias bellottii* (present study), origi-nal description (Vizcaino 1992) and type material. Abbreviations: Max = maximum L=length, W=width, * not measured, *2 material in poor stage of conservation.

		Males			Females		
Host		***A. bellottii***	***C. paleatus***	***C. paleatus***	***A. bellottii***	***C. paleatus***	***C. paleatus***
Source		**Present study**	**Vizcaíno 1992**	**Type Material (present study)**	**Present study**	**Vizcaíno 1992**	**Type material (present study)**
Total length		1322 (1043–2897)	2071 (895–6013)	2044 (938–4831)	5543 (4256–7076)	3081 (637–7505)	4347
Max. width		392 (245–558)	215 (95–427)	214 (127–388)	604 (521–673)	251 (95–494)	341
Proboscis	L	98 (89–114)	111 (95–171)	*2	89 (77–101)	121 (94–152)	235
	W	62 (52–75)	81 (57–105)	*2	70 (66–73)	86 (76–105)	247
Anterior circle of hooks	L	24 (23–26)	27 (25–29)	*2	27 (25–29)	31 (27–35)	*2
	W	5 (4–5)	5 (5–7)	*2	6 (5–8)	7 (5–8)	*2
Middle circle of hooks	L	25 (16–27)	26 (24–28)	*2	26 (25–27)	25 (23–28)	*2
	W	5 (4–5)	6 (5–7)	*2	5 (5–6)	5 (5–8)	*2
Posterior circle of hooks	L	18 (15–21)	21 (19–23)	*2	15 (14–17)	21 (19–30)	*2
	W	4 (3–4)	5 (5–5)	*2	4 (3–5)	5 (4–6)	*2
Long lemnisci	L	254 (157–375)	*	217 (142–416)	372 (326418)	*	137
	W	64 (32–89)	*	101 (32–223)	66 (51–77)	*	98
Short lemnisci	L	237 (168–311)	*	176 (128–307)	287 (159–372)	*	88
	W	54 (33–81)	*	87 (38–155)	67 (61–72)	*	98
Proboscis receptacle	L	153 (127–179)	206 (133–295)	100 (62–144)	191 (177–212)	159 (162–285)	*2
	W	74 (66–84)	86 (57–114)	74 (50–94)	88 (83–91)	88 (75–109)	*2
Anterior testis	L	529 (400–777)	255 (75–855)	277 (137–774)	—	—	—
	W	246 (169–414)	145 (38–295)	155 (95–290)	—	—	—
Posterior testis	L	410 (281–699)	220 (68–741)	242 (101–652)	—	—	—
	W	218 (144–307)	144 (41–266)	133 (75–251)	—	—	—
Cement gland	L	425 (292–673)	184 (53–456)	236 (68–517)	—	—	—
	W	212 (118–299)	122 (60–219)	131 (82–263)	—	—	—
Cement reservoir	L	157 (113–229)	102 (23–209)	113 (44–242)	—	—	—
	W	120 (79–170)	92 (34–190)	99 (55–173)	—	—	—
Saefftiggen's pouch	L	342 (241–452)	204 (94–428)	226 (137–488)	—	—	—
	W	127 (93–162)	70 (23–143)	74 (42–118)	—	—	—
Everted copulatory pouch	L	165 (115–211)	*	*2	—	—	—
	W	126 (98–178)	*	*2	—	—	—
Eggs	L	—	—	—	28 (22–33)	32 (30–34)	29 (26–31)
	W	—	—	—	8 (6–10)	15 (14–15)	13 (12–15)
Embryo		—	—	—	*	26 (25–27)	*2
Genital complex		—	—	—	*	256 (26–428)	*2

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